ABSTRACT

Charles University in Prague, Faculty of Pharmacy in Hradec Králové
Department of Analytical Chemistry
Candidate: Mgr. Jiří Plíšek
Supervisor: prof. RNDr. Petr Solich, CSc.
Supervisor specialist: assoc. prof. RNDr. Dagmar Solichová, Ph.D.
Title of dissertation thesis: Using of separation methods in clinical research

This thesis is devoted to development and validation of new LC methods for the determination of biologically active substances, specifically a fat-soluble vitamins (vitamin A, E and D) and theirs metabolites. Vitamins are essential micronutrients which cannot be synthesized by human organism with the exception of steroid hormone precursor of vitamin D. Vitamins are involved in biochemical processes of organism directly or as coenzymes. Interest in the clinical monitoring of these substances is increasing constantly over the last years.

It needs to use modern instrumental techniques such as the UHPLC coupled with MS detection for the determination of vitamin D metabolites (25-hydroxy vitamin D_3 and 25-hydroxy vitamin D_2) due to their low concentrations in serum. The use of classical liquid chromatography with diode array detection is suitable in case of monitoring of levels of vitamin A (retinol) and E (α-tocopherol) in breast milk and vitamin E (α-tocopherol) in red blood cell membranes. The sample pretreatment procedure has to be included prior to analysis whereas the object of interest is a complex biological matrix. This stage is crucial for the determination of analytes, as it has a direct impact on the success of whole analysis, moreover its high quality and accurate performance will prolong the instrumental lifetime. Dissertation thesis therefore includes a comprehensive view at the investigation process, ie. the sample collection, storage and sample preparation within the preanalytical phase, over the analytical phase, through to processing and interpretation of the results in the postanalytical phase.

The experimental section is divided into four thematic parts. The first two parts of theses describes the determination of retinol and α-tocopherol in human breast milk using monolithic columns and columns with porous shell particles. The comparison of these columns, their advantages and disadvantages are discussed in the part of optimization and validation of the method. The following part is devoted to the analysis and clinical monitoring of 25-hydroxy vitamin D_3 and 25-hydroxy vitamin D_2 using UHPLC tandem mass spectrometry method. The final section of theses describes the preferential

combination of ultracentrifugation and solid phase extraction followed by chromatographic analysis of α-tocopherol in the membrane of human erythrocytes.

All aforementioned methods were developed and validated in the Research Laboratory at 3rd Internal Gerontometabolic Clinic at the University Hospital Hradec Králové and successfully published in analytically oriented international journals with impact factor.